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16. ABSTRACT

The work reported here focuses on the effects of acid deposition on nitrification, denitrification and N_2 fixation associated with plant roots and nitrification and denitrification in surface soil. Simulated acid precipitation of pH 2.0, 3.0, 4.0 and 5.6 was employed. The purpose of the extreme treatments (pH 2.0 and 3) was not to directly simulate ambient rates but rather to identify thresholds at which effects may begin to occur. It was then attempted to assess whether these thresholds may be reached in natural terrestrial systems. Results were reported from studies designed to identify physical/chemical mechanisms that may mediate effects of acid deposition on soil microbial activity. The experiments reported here are considered to be preliminary studies intended largely to identify possible "pressure-points" and mechanisms that should be investigated more thoroughly.

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CHAPTER 5

Microbial Response to Acid Deposition and Effects on Plant Productivity

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Plant productivity may be altered profoundly if terrestrial acid deposition affects soil microbial activity. Soil microorganisms are largely responsible for the cycling of nitrogen and sulfur in ecosystems. Since N is the major limiting nutrient in natural terrestrial systems, changes in rates of microbial N transformations could alter the primary productivity of these systems. The major storage form of N is as organic matter, and the nutrient is released for plant utilization through mineralization by microbes. The availability of nitrogen to plants is also a function of the oxidation state; for substantial plant uptake to occur, N must be as NO_3^- or NH_4^+, with specific plants usually preferring one or the other species. Oxidation of NH_4^+ to NO_3^- is catalyzed by soil bacteria during nitrification. Most nitrogen enters the ecosystem through bacterial reduction of N_2 to NH_4^+ (N_2 fixation). Conversely, N is lost from the soil through anaerobic bacterial reduction of NO_3^- to the gaseous species N_2 and N_2O (denitrification).

Among microbial nitrogen transformations, nitrification has been predicted to be one of the processes more sensitive to acid precipitation [1,2] because it is generally believed to be catalyzed by a relatively limited diversity of autotrophic nitrifiers (known to be acid-sensitive on laboratory media). Strayer et al. [3] examined the effects of accelerated acidification on nitrification in surface soil from columns and obtained interesting but complex results. When high NH_4^+ amendments were added to the nitrification assay, all acid treatments tested (pH 3.3–4.1) caused substantial reductions in nitrification rates. However, when NH_4^+ was not added to the soil, the acid treatments generally caused no detectable effect and in some

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cases caused a slight stimulation in NO_3^- production. These results suggest that in soils with low natural concentrations of NH_4^+ (such as many forest soils) short-term acid deposition may not substantially affect nitrification. The work by Strayer et al. [3] is also consistent with the occurrence of heterotrophic nitrifying organisms in naturally acid forest soils, these heterotrophic nitrifiers being less sensitive to acidity than autotrophic nitrifiers [4,5]. Examining soil exposed to acid rain (pH 3.0) from a coking works for one year, Wainwright [6] found essentially no effect on nitrifying activity.

There has also been concern that symbiotic N_2 fixation may be adversely affected by acid precipitation. Shriner and Johnston [7] found that simulated rain of pH 3.2 applied for 1–9 weeks caused decreased nodulation in kidney beans. Potential effects on a microbial–plant symbiosis can be complex in that they can be mediated through effects on the plant or on the soil.

Of the three microbial nitrogen transformations discussed here, denitrification might be expected to be the least sensitive to acid precipitation simply because it is catalyzed by a relatively diverse group of heterotrophic microbes. While the process is known to be inhibited in extremely acid soils, the overall rates of the reaction have not been found to be particularly sensitive to slight shifts in soil pH [8]. Slight soil acidification does, however, alter the composition of the products formed, causing increased N_2O production relative to N_2 [9].

Distribution of heterotrophic microbial activity in soil reflects the availability of organic carbon. While most types of microbial activity occur to some extent throughout the soil profile, the recognition that maximal activity commonly occurs in somewhat discrete areas of the soil is important to understanding the potential effects of acid deposition. Maximum carbon availability and, hence, maximum heterotrophic activity is usually found near plant roots and in the upper portion of the soil profile, reflecting the site of initial deposition of plant material.

The work reported here focuses on the effects of acid deposition on nitrification, denitrification and N_2 fixation associated with plant roots and nitrification and denitrification in surface soil. We employed simulated acid precipitation of pH 2.0, 3.0, 4.0 and 5.6. The purpose of the extreme treatments (pH 2.0 and 3.0) was not to directly simulate ambient rates but rather to identify thresholds at which effects may begin to occur. We then attempted to assess whether these thresholds may be reached in natural terrestrial systems. We also report results from studies designed to identify physical/chemical mechanisms that may mediate effects of acid deposition on soil microbial activity. The experiments reported here are considered to be preliminary studies intended largely to identify possible "pressure-points" and mechanisms that should be investigated more thoroughly.

APPROACH

Microbial Activity in Rhizosphere Soil

Subterranean clover and Briggs variety barley were grown from seed in a Reiff soil that had been sieved (<4 mm) but not dried before use (Table I). The

Table I. Soil Characteristics

Series	*Classification*	*Texture*	pH	*% Organic Matter*	*CEC (meq-100 g^{-1})*	*Base Saturation (meq-100 g^{-1})*
Reiff	Typic xerorthent	Sandy loam	7.4	n.d.	18	18
Shaver	Pachic xerumbrept	Sandy loam	6.4	5.2	19	13
Surnuf (sub surface) sample)	Xeric haplohumult	Silt loam	5.3	0.2	27	15
Aiken	Xeric haplohumult	Loam	5.3	3.7	20	12

clover seed was treated with commercial rhizobium inoculant and seeds planted in 6-in.-diameter pots lined with plastic. Plant–soil systems and unplanted soils were spray-irrigated under four treatment regimes (pH 2.0, 3.0, 4.0 and 5.6) at a rate of 250 mL three times per two weeks to total about 13.2 cm of precipitation over ten weeks. The ionic composition of the treatment solution reflected that previously monitored in northern California [10], including a quantity of H_2SO_4 and HNO_3 (2:3 on an equivalent basis) to yield the specified pH plus a standard salt solution consisting of Mg^{2+}, 6; Ca^{2+}, 7; NH_4^+, 15; Cl^-, 15; K^+, 1.5 $\mu eq\text{-}L^{-1}$. Plants and soils were harvested after 10–12 weeks of treatment for microbial activity assays and nutrient analyses.

Nitrification activity was determined using a modification of the method described by Belser and Mays [11]. Soil samples (5 g) were leached at a constant rate for 7 h on a mechanical extractor with a solution containing NH_4^+ and ClO_3^-. Nitrite production was determined at 2-h intervals using a standard diazotization colorimetric method. Denitrification activity was determined using the acetylene inhibition technique as described by Smith et al. [12]. Nitrous oxide accumulation was quantified by gas chromatography (GC) during incubation periods of less than 8 h. A standard acetylene reduction assay was used to determine the N_2 fixation activity associated with clover roots. The assay was initiated immediately after the root material was harvested and ethylene production was quantified by GC. Soil pH was determined using a 1:1 soil-water slurry.

Four replicate pots were used for each treatment; each pot contained eight plants. Rates of microbial activity were determined by linear regression. When analyses of variance indicated significant differences among treatment means, a Student–Newman–Keuls (SNK) multiple range test was employed [13].

Microbial Activity in Surface Soil

Effects of simulated acid precipitation on activity in surface soil was investigated in five different plant-soil systems. All soils were sieved (<4 mm) and packed in 15-cm-diameter, 30-cm-long plastic cylinders. Shaver soil was planted with soft chess and ponderosa pine seedlings; Reiff soil was planted with clover or barley, or left unplanted. For each plant–soil combination, a set of six replicates was run with and without the addition of fertilizer N and S (N as NH_4NO_3 at 200 $kg\text{-}ha^{-1}$; S as $CaSO_4 \cdot 2H_2O$ at 70 $kg\text{-}ha^{-1}$). The composition of the acid rain, the application rate and the treatment duration were similar to those described in the preceding section. For each plant–soil set, nitrification activity was determined in the top 1 cm of soil. Nitrification and denitrification activities were determined in the surface 1 cm as well as in the bulk soil from which the surface layer had been removed for the Shaver soil containing two-year-old seedlings of ponderosa pine.

Exchangeable NH_4^+ in soil was determined on a 1 *N* KCl extract using an ammonia electrode. Nitrate was determined by reduction to nitrite with hydrazine sulfate and subsequent diazotization.

Aluminum Mobilization and Toxicity

Five-gram samples of 26 soils were leached with 50 mL of treatment solution over a 2-h period. Treatment solutions of eight pH values were used and the resulting leachates were analyzed for pH and cation content. Acid digests of the leachates were analyzed for Mn and Al by atomic absorption. Leachates from three soils were chosen as being representative of the ranges of Al and Mn content and used for bioassays.

Wells 1-cm in diameter were cored in 1-cm-deep agar plates (Czapek Dox-Oxoid), which were uniformly inoculated with a spore suspension of *Aspergillus flavus*. Soil leachates (0.7 mL) from the pH 2, 3, 4 and 5.5 treatments were added to the center wells. Zones of inhibition of spore germination were first qualitatively documented through visual inspection. Inhibition of spore germination was apparent only from leachates of the pH 2 treatments. To quantify inhibition, the experiment was rerun with the leachates from the pH 2, 3 and 4 inputs and respiration was determined on cores from the plates by GC quantification of CO_2 production. Five replicate flasks were run for each soil-treatment set, each flask containing ten cores (five from each of two plates). For each plate, two sets of cores were tested: one set taken from within the zone of inhibition and one from outside the zone. Bioassays were also run on plates containing the following solutions: 1.0 *M* acetate–HCl buffer, pH 3.0; buffer (pH 3.0 as before) containing 32 $\mu g\text{-}mL^{-1}$ Al and 20 $\mu g\text{-}mL^{-1}$ Mn; and buffer solutions containing the same concentration of each metal separately. All Al-containing solutions (soil leachates and buffers) were titrated with 1.0 *N* NaF until free F^- was just detectable by a fluoride-specific electrode. These solutions were also used for parallel assays.

RESULTS AND DISCUSSION

Root-Associated Microbial Activity

Nitrogen fixation activity by clover roots was significantly greater in plants receiving the extreme pH 2 treatment (Table II). This may have been due to the fertilizer

Table II. Acetylene Reduction by Clover Roots

Input pH	C_2H_4 ($nmol\text{-}g^{-1} - h^{-1}$)
2.0	320a*
3.0	33.2b
4.0	27.7b
5.6	60.2b

*Different letters indicate difference between means at 5%, SNK multiple-range test.

value of the N and S inputs, since in a subsequent experiment in which N and S fertilizers were included there were no significant differences in clover root fixation activity with the different pH inputs.

While there was a pattern of lower nitrification activity with extremely low pH inputs, there were no statistically significant differences (at 5%) associated with input pH in the unplanted or clover rhizosphere soil (Figure 1). In barley rhizosphere soil receiving pH 2 input, nitrification activity was significantly lower. In general, nitrification activity was lower in rhizosphere soil than in unplanted soil and this was significant in two cases (clover, pH 3 and barley, pH 2).

Denitrification activity was significantly higher in barley soil receiving pH 2 treatments but was not significantly different across input pH in clover soil. In the presence of both clover and barley roots, denitrification was substantially greater than that in unplanted soil in which activity was virtually undetectable over the period of assay.

In each plant–soil system the pH of the soil was lower with the extreme acid inputs compared to pH 4.0 and 5.6 inputs. However, the presence of the plant roots also significantly affected the pH of the soil. The presence of clover roots appeared to acidify the rhizosphere soil, and the effect of barley roots was similar. The extent of root influence on soil pH was related to the mass of roots present in the soil. In general, the greater the root mass, the greater was its acidifying effect. Plant mass (root plus shoot weight) was greater in the systems receiving pH inputs of 2 and 3. This increased plant productivity probably resulted largely from the fertilizer value of the acids HNO_3 and H_2SO_4, but also perhaps from increased nutrient availability resulting from slight acidification of the initially neutral soil. Nutrient analysis of the plant material indicated significantly greater S and P concentrations in clover exposed to pH 2 input and greater N concentrations with pH 2 and 3 inputs. Similar patterns were found with barley. Subsequent experiments in which fertilizer N and S were added to the soils substantiate this interpretation of these results (data not shown).

It is well known that plant roots affect the pH of associated soil through differential ion uptake, respiratory CO_2 production and organic acid production [14]. In the experiments reported here, simulated acid precipitation lowered the pH of the bulk soil only when extreme pH 2 treatments were used. However, the presence of plant roots significantly affected the soil pH in five out of eight cases. If one then considers the potentially acidifying effect of realistic quantities of acid precipitation, it seems likely that in rhizosphere soil the influence of plant roots would exceed that of acid precipitation. However, the short-term nature of these experiments should be borne in mind.

The data on microbial activity also reflects the more direct influences of roots on soil microbial processes. Root exudates and sloughed materials provide carbon inputs to root-associated heterotrophs [14]. This can be seen in the high rates of denitrification (a carbon-dependent process) associated with clover and barley roots as compared to unplanted soils. The lower rates of nitrification (an autotrophic process) associated with increased root mass (and increased acid input) is also consistent with a root-originating carbon effect. Thus, through mechanisms

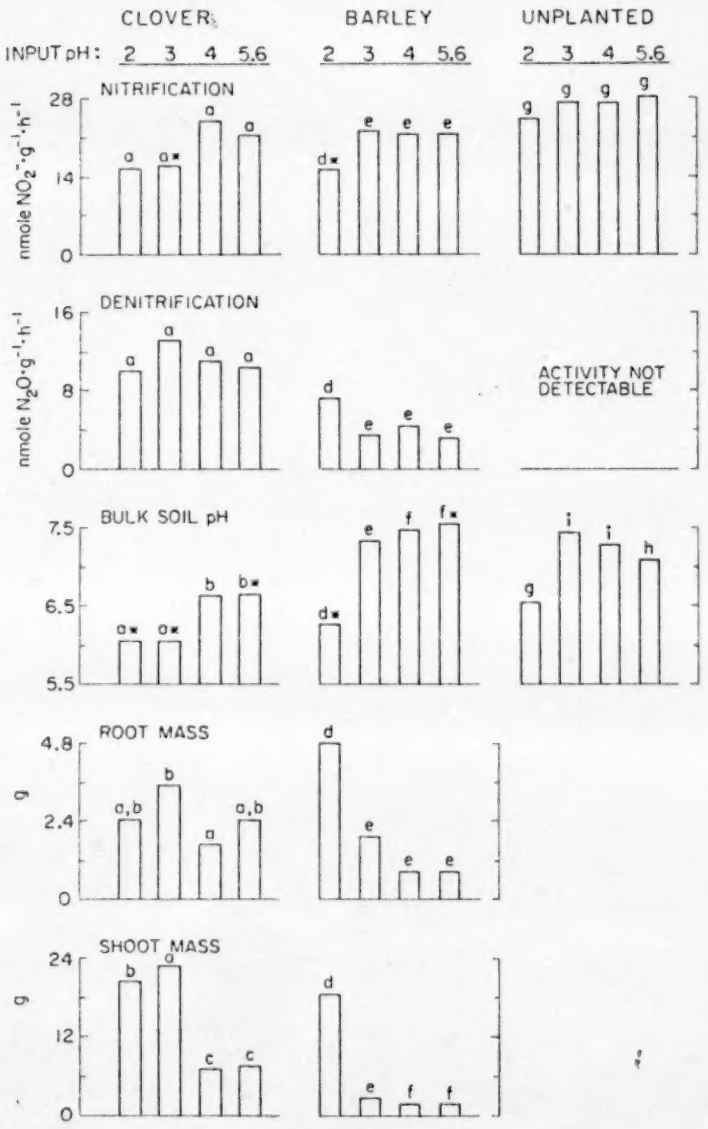

Figure 1. Effects of simulated acid precipitation on microbial activities and plant productivity in a Reiff soil. Within a planting regime, different letters indicate differences between means at 5%, SNK multiple-range test. Differences between a treatment mean in planted soil vs unplanted soil (5%, by Student's t-test) are indicated by.*

of carbon supply, the influence of roots on associated microbial activity may be substantially greater than that of current rates of acid precipitation. Despite the short duration of these exposures, the results reported suggest that microbial activity in rhizosphere soil may be relatively resistant to direct effects of acid precipitation. Unlike rhizosphere soil, the surface soil should be more directly affected by acid precipitation; hence, microbial activity in this zone may be modified.

Microbial Activity in Surface Soil

The effects of simulated acid precipitation on nitrification and denitrification in the surface 1 cm of a Shaver soil containing ponderosa pine seedlings is shown in Fig. 2. It should be noted that the rooting system of a tree seedling is much less extensive than that of a grass or a clover plant; hence, substantial rhizosphere effects are much less likely. In the surface layer of this granitic soil, nitrification activity was significantly depressed with pH 2 treatments, but enhanced in soil from lower in the profile. Determination of exchangeable NH_4^+ indicated that nitrification in surface soil was not inhibited by H^+ mobilization of NH_4^+ cations from the surface soil to lower depths.

The effects on denitrification were almost the reverse of those on nitrification. Denitrification activity was significantly greater in surface soil receiving the extreme input of pH 2. The process of denitrification requires both available NO_3^- and carbon (as well as O_2 limitation). Increased NO_3^- availability resulting from the higher HNO_3 inputs apparently had not stimulated the process in surface soil; nitrate analysis indicated that while the NO_3^- concentration was greater in surface soil receiving pH 2 input, it was similarly greater lower in the profile. Through the use of sterile controls, we also determined that the increased rate of N_2O evolution in soil receiving pH 2 treatment was not due to acid-stimulated chemical decomposition of HNO_2 (data not shown). It is plausible that denitrification activity in surface soil was stimulated by increased carbon availability from soil organic matter which was chemically altered by the pH 2 input and resulting soil acidification. Other work in our laboratory supports this mechanism of effect, but at this time it remains a "working hypothesis."

It is not surprising that the effect of the acid inputs on soil pH was greater in the surface 1 cm than in the bulk soil. The substantial pH decrease (from 6.5 to 3.8) caused by the extreme pH 2 input most likely caused the reduction in nitrification observed in the surface soil. But it should be noted that the less pronounced pH decrease in the lower soil (from 6.5 to 5.4) was not associated with a decrease in nitrifying activity but rather an increase. Interpretation of the nitrification data presented here is facilitated by the results from the eight other plant–soil–fertilizer systems that were studied simultaneously (data not shown). In only one other instance (a fertilized Reiff soil planted in barley) was nitrification activity in surface soil significantly affected by the pH 2 input. In the seven cases in which activity was not significantly altered, the decrease in soil pH resulting from the acid input was much less substantial than that reported here or observed in the

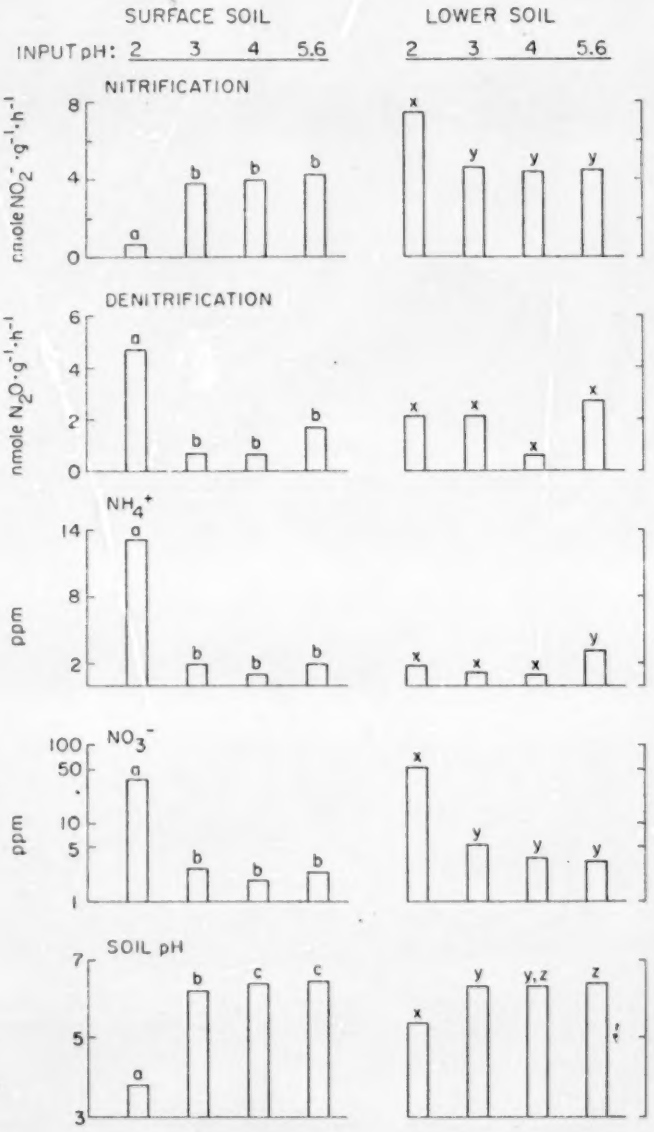

Figure 2. Effects of simulated acid precipitation on nitrification, denitrification and nitrogen species in a Shaver soil planted with ponderosa pine.

Reiff–barley system. Thus, it appears that substantial decreases in pH of surface soil will inhibit nitrification. The critical question then becomes one of the magnitude and rate of soil acidification that may result from realistic or ambient rates of acid precipitation. While the susceptibility of a soil to acidification varies as a relatively complex function of the soil buffering capacity, the degree of acidification caused here by pH 2 treatments should be expected to be uncommon in natural soil systems receiving acid rain.

It is not surprising that acid inputs will affect soil microbial activity (at least on a short-term basis) if there is a substantial decrease in the pH of the soil solution. A somewhat more complex and intriguing question is whether soil microbial activity can be altered by acid precipitation that does not cause a discernible decrease in soil pH. One can hypothesize several mechanisms through which microbes might be affected without a measurable pH shift; two such mechanisms pertain to buffering species in soil. Protonation of soil organic matter functional groups could (perhaps transiently) alter organic matter availability to microbes. Work in our laboratory is addressing this question. Second, aluminum-containing minerals can also buffer soil solution pH. While the mineral species involved are numerous and exact reaction mechanisms complex, the net consumption of H^+ by these minerals usually involves the solubilization or mobilization of $Al^{3+}(OH)_x(H_2O)_y$ forms. The solubilization of Al can be a transient phenomenon with reprecipitation and polymerization reactions rapidly occurring. Can mobilization of aluminum (or manganese) resulting from acid precipitation cause toxicity to soil microbes?

Aluminum Mobilization and Toxicity

The compositions of the leachates used for bioassays are given in Table III. With increasing acidity of the input solution, increasing quantities of Al and Mn were eluted. Between soils, however, the quantity of Al/Mn mobilized was not simply a function of the output solution pH. While the Surnuf soil consistently had the most acid leachate pH, less Al and Mn were eluted than from the two other soils. As previously described in the Approach section, these test solutions were obtained by rapidly leaching a small quantity of soil with a relatively large volume of solution. This is roughly comparable to about 8.9 cm of precipitation input moving through 1 cm of soil in 2 h. The quantities of Al/Mn in the leachates may be unrealistically low due to the short period allowed for Al/Mn solubilization and large volume of solution. Similarly, the leachate period was short for reprecipitation and polymerization reactions to go to completion. However, the question under investigation was one of transient mobilization, not whether Al was mobilized totally out of a soil profile. The problems associated with this type of technique should be borne in mind as the results of the bioassays are discussed.

There were no visually significant effects of leachates from soils treated with pH 5.6, 4.0 or 3.0 solutions on germination of *Aspergillus flavus* spores. Leachates from the Shaver and Aiken soils receiving pH 2.0 inputs did significantly inhibit

Table III. Aluminum and Manganese Content (μg-mL^{-1} of Leachate) of Soil Leachates

Input pH	*Parameter*	*Soil Series*		
		Surnuf	*Shaver*[a]	*Aiken*
5.6	Leachate pH	5.0	6.9	5.9
	Al	0.039	0.146	0.140
	Mn	0.001	0.574	0.003
4.0	Leachate pH	4.9	6.8	5.9
	Al	0.010	0.188	0.010
	Mn	0.003	0.644	0.003
3.0	Leachate pH	4.5	6.7	5.8
	Al	0.04	0.12	0.21
	Mn	0.02	2.46	3.70
2.0	Leachate pH	2.7	3.0	3.1
	Al	0.99	32.4	16.1
	Mn	0.16	20.1	25.8

[a] This Shaver series soil is different from that characterized in Table I. This sample had an initial pH of 5.7 and percentage of organic matter of 3.1.

spore germination (Table IV). To determine if unidentified species present in the soil leachates were involved in the observed toxicity, solutions buffered to pH 3.0 and containing Al and Mn concentrations similar to those of the Shaver soil leachates were assayed. While the "leachate simulations" did significantly inhibit spore germination, they were not as toxic as the Shaver and Aiken (high Al/

Table IV. Effects of Soil Leachates and "Simulated Leachates" on *A. flavus* Spore Germination

Input pH	*Soil Leached*		
	Surnuf	*Shaver*	*Aiken*
4.0	98.8 ± 11.2[a]	108.2 ± 13.6	96.1 ± 8.4
3.0	111.4 ± 14.8	99.6 ± 6.6	100.3 ± 10.1
2.0	115.3 ± 16.5	5.8 ± 2.7	17.7 ± 4.0
2.0 + F^-	105.2 ± 12.0	85.4 ± 10.3	92.0 ± 7.9

	Simulated Leachates[b]			
	pH 3 + Al + Mn	pH 3 + Al	pH 3 + Mn	pH 3
Buffer ± Metals	34.4 ± 8.2	39.2 ± 9.5	103.2 ± 8.1	108.0 ± 14.3
Solutions + F^-	88.1 ± 6.0	82.6 ± 9.8		

[a] Values given are 100 × [(respiration rates from zones of inhibition)/(respiration rates from unaffected zones of same plates)], ± standard deviation.

[b] 1.0 *M* acetate-HCl buffer, pH 3, with or without added Al (32 μg-mL^{-1}) and Mn (20 μg-mL^{-1}).

Mn) leachates. This indicates that some unidentified component(s) (perhaps organic ligands) were enhancing leachate toxicity. The Al and Mn were then factored out and the results indicated that the "simulated leachate" toxicity resulted from the Al present in solution. To further substantiate this point, Al-containing solutions were titrated with fluoride as described in the Approach section. When these fluoride-amended leachates and "simulated leachates" were tested, the presence of this strong Al^{+} complexing species significantly reduced the solution toxicity. This indicates that a major portion of the leachate effect resulted from Al toxicity.

Solution concentrations of 16 and 32 $\mu g\text{-}mL^{-1}$ Al in leachates from the Aiken and Shaver soils were found to be toxic to *Aspergillus flavus* spore germination; the leachate from the Surnuf soil containing 1 $\mu g\text{-}mL^{-1}$ Al was not toxic. This is consistent with work by Ko and Hora [15], in which a threshold Al toxicity of 0.65 ppm was found for ascospore germination in solution. Other soil microbial processes may be more or less sensitive to Al; fungal spore germination is generally considered to be relatively sensitive to Al toxicity.

The concentrations of Mn found in the soil leachates tested (0.1–26 $\mu g\text{-}mL^{-1}$) were not found to cause toxicity. Dobereiner [16] reported that additions of 40 ppm Mn to acid soils were inhibitory to symbiotic N_2 fixation. The highest Mn concentration tested in our work (26 $\mu g\text{-}mL^{-1}$) was lower than that used by Dobereiner.

We have observed microbial Al toxicity only in soil leachates resulting from an extreme acid input of pH 2. However, our data confirm that soils vary substantially in susceptibility for Al and Mn mobilization; the possibility remains that toxic levels of Al may be mobilized by less acidic inputs. Potential toxicity to microbes in terrestrial systems depends on the quantities of Al and/or Mn that may be mobilized by ambient acid precipitation.

CONCLUSIONS

There were no observable effects on the microbial activities tested of the acidified precipitation (pH 4.0) that was most similar to that of "real" acid rain. However, the short durations of the treatments (3 months) precludes statement of direct conclusions concerning effects of long-term, ambient acid deposition. Substantially intensified acid inputs (pH 2 and in some cases pH 3) did affect microbial activity. The total H^{+} load from three months of pH 2 precipitation would be the same as that from 30 months of pH 3 or 300 months of pH 4. However, the effects that result from pH 2 precipitation cannot be extrapolated to predict long-term effects of more realistic acidified precipitation because the interactions of acidity with the soil physical and microbial components have an extremely important time dependence. Mineral solubilization reactions (involved in buffering), microbial adaptation and reequilibration of organic matter pools are slow processes. Effects which result from accelerated treatments may never occur with less rapid rates of H^{+} input. But the possibility that changes in microbial activity will result from long-term, "moderate"-pH inputs makes continued investigation, using longer exposure periods, essential.

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